Award Number:  DAMD17-01-1-0132

TITLE:  To Ascertained Distinctive Gene Expression Patterns for the Prediction of Docetaxel (Taxotere) Chemosensitivity or Chemoresistance in Human Breast Cancer

PRINCIPAL INVESTIGATOR:  Jenny C. Chang, M.D.

CONTRACTING ORGANIZATION:  Baylor College of Medicine
Houston, Texas 77030

REPORT DATE:  October 2002

TYPE OF REPORT:  Annual

PREPARED FOR:  U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland  21702-5012

DISTRIBUTION STATEMENT:  Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
To Ascertain Distinctive Gene Expression Patterns for the Prediction of Docetaxel (Taxotere) Chemosensitivity or Chemoresistance in Human Breast Cancer

Jenny C. Chang, M.D.

Baylor College of Medicine
Houston, Texas 77030
E-Mail: jcchang@breastcenter.tmc.edu

Chemotherapy is of proven benefit in reducing the risk of death for a subset of patients with early breast cancer, but doctors have problems deciding exactly who should receive this therapy, and which therapy will be most effective for a given patient. As a result, some patients needlessly receive chemotherapy. Even in those patients who clearly require chemotherapy, doctors cannot identify those patients whose tumors might not be responsive to a particular chemotherapy drug. Chemotherapy is also associated with high costs and toxicity including nausea, vomiting, damage to nerves, etc. and increased risk of infections that are sometimes life threatening. The emerging cDNA array technology provides a means to comprehensively appreciate genetic variations in different breast tumors, and may be utilized as a test for chemotherapy sensitivity. Taxotere has one of the highest response rates in breast cancer, and is widely prescribed for the treatment of breast cancer. The aims of this study are therefore, to investigate and validate differential gene expression patterns from core biopsies from patients whose breast tumors either shrunk after Taxotere chemotherapy, or failed to respond. These genes whose expression patterns are associated with Taxotere response could be used to create a simple test of predictor genes to help doctors treat breast cancer more effectively.
# Table of Contents

Cover...........................................................................................................1

SF 298..........................................................................................................2

Introduction.................................................................................................4

Body.............................................................................................................5

Key Research Accomplishments.................................................................6

Reportable Outcomes..................................................................................7

Conclusions................................................................................................7

References.....................................................................................................7

Appendices...................................................................................................
Introduction

Optimal systemic treatment after breast cancer is the most crucial factor in reducing mortality in women with breast cancer. Adjuvant chemotherapy and hormonal treatment both reduce the risk of death in breast cancer patients. However, while estrogen receptors status predicts for response to hormone treatments, there are no clinically useful predictive markers for chemotherapy response. All eligible women are therefore treated in the same manner, even though de novo drug resistance will result in treatment failures in many breast cancer patients. Currently, there are no methods available to distinguish those patients who are likely to respond to specific chemotherapies, and given the accepted practice of prescribing adjuvant treatment to most patients, even if the average expected benefit is slow, the selection of appropriate patients represents a major advance in the clinical management of breast cancer today.

We therefore set out to identify gene expression patterns in primary breast cancer specimens that might predict response to Taxotere. Neoadjuvant chemotherapy allows for the sampling of the primary tumor for gene expression analysis and for direct assessment of response to chemotherapy by following changes in tumor size during the first few months of treatment. Hence, neoadjuvant chemotherapy provides an idea platform to rapidly discover predictive markers of chemotherapy response.
In this present study, we hypothesize that high throughput quantitation of gene expression, it is possible to assess thousands of genes simultaneously, and that these expression patterns in different breast cancers might correlate with and thereby predict response to treatment. The purpose of this study was to (1) demonstrate that sufficient RNA could be obtained from core biopsies to access gene expression, (2) to identify groups of genes that could be used to distinguish primary breast cancers to responsive or resistance to Taxotere, and (3) to identify gene pathways that could be important in a mechanism of action of taxotere.

Body of Research

From September 17, 2001 to September 16, 2002, we have recruited 42 patients with locally advanced breast cancer. Core biopsies were obtained from the primary breast cancers before commencement of neoadjuvant chemotherapy. Clinical responses before and after four cycles of chemotherapy were measured in all primary breast cancers.

A total of 6 core biopsies were obtained from each primary cancer. Two core biopsy specimens were transferred immediately to liquid nitrogen and snap frozen at −80° C. Each core biopsy measured approximately 1 cm x 1 mm. As these core biopsies were too small for micro dissection, we ascertained the tumor cellularity of the pretreatment core biopsies. In general, the core biopsies showed good tumor cellularity with median tumor cellularity of 75% (range 40-100%). Each core biopsy yielded 3-6 mg of total RNA, which is more than sufficient to generate approximately 20 mg of label cRNA needed for hybridization with the Affymetrix U95Av2 Genechip, using the manufacturer’s standard protocols. To date, we have analyzed 24 out of the 42 collected core biopsy specimens. We have begun initial exploratory analysis. We compared
the expression data in sensitive and resistant tumors to identify gene significance differentially expressed between the two groups. We applied filtering to eliminate genes with uniformly low expression or whose expression did not vary significantly across the samples retaining approximately 1,600 genes. We then applied t-test after log transformation, to select discriminatory genes. To date, we have selected 92, 300, 551 genes as differentially expresses at p values of 0.001, 0.01, 0.05, respectively.

In the 92 gene list, among the genes overexpressed in the resistant cluster, most are involved in transcriptional regulation, signal transduction, or have unknown functions. In the sensitive tumors, some are involved in signal transduction and cell cycle, cytoskeleton and adhesion processes, protein transport, protein modification, stress and apoptosis or have unknown functions. We recently begun to confirm the expression measurements obtained from the affymetrics chips with values from semiquantitative RT-PCR. We have done 15 genes and compared their measurements with QRT PCR. Significantly correlation was seen between the two methods.

**Key Research Accomplishments**

Three abstracts have been submitted and accepted for international meetings. Two were submitted to the San Antonio Breast Cancer Symposium in 2001 and 2002. This abstract was also submitted to the ASCO meeting in Florida in 2002. This was selected for oral presentation. A manuscript is underway to discuss the preliminary findings of this study. This study was also selected for a plenary presentation in the Era of Hope Meeting in Florida in 2002.
Reportable Outcomes


Conclusions

We have determined that 1) sufficient RNA can be obtained from core needle biopsies to hybridize Affymetrix GeneChips for assessment of gene expression patterns 2) differential gene expression patterns exist that can distinguish resistant versus sensitive tumors. We will continue our current experiments to further increase patient recruitment to define and refine patterns of resistance and sensitivity.

References